

CLAIMS

1. A compound comprising a polysaccharide acid a pendant moiety linked at least one terminal unit derived from a sialic acid unit which includes a functional group selected from N-maleimide groups,
 5 vinylsulphone groups, N-iodoacetamide groups orthopyridyl disulphide groups.

2. A compound according to claim 1 in which the pendant moiety is linked at the reducing terminal unit of the polysaccharide.

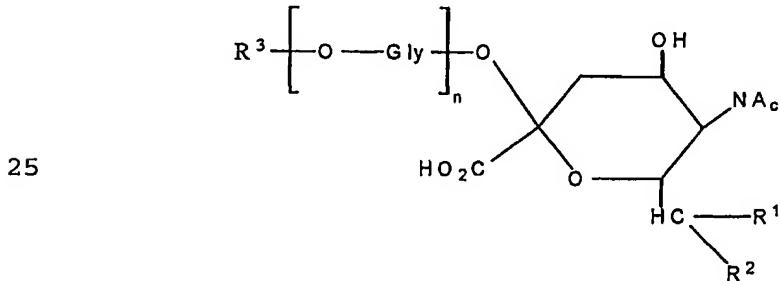
3. A compound according to claim 1 or claim 2 in which the
 10 moiety is linked at the non-reducing terminal unit of the polysaccharide.

4. A compound according to any preceding claim in which the moiety comprises an alkanediyl group and/or an arylene group and a linkage optionally in combination with a oxalkylene or oligoaxa-alkylene group which is a secondary amine linkage, a hydrazone, an alkyl hydrazide linkage or a
 15 peptide linkage.

5. A compound according to any preceding claim in which the functional group is N-maleimido.

6. A compound according to any preceding claim in which the polysaccharide is a polysialic acid, preferably consisting substantially only of
 20 sialic acid units

7. A compound which the compound has the formula



in which one of the following groups of definitions apply:

i) R¹ is H or -CHOHCH₂OH, R² is OH and R³ is either
 30 -CH₂CHR⁴R⁵ or -CH(CH₂OH)CHR⁴R⁵ in which R⁴ and R⁵ together represent
 =N-NR⁶ or R⁴ is H and R⁵ is -NR⁶R⁷ in which R⁶ is an organic group

comprising the said functional group or is H, and R⁷ is H or R⁶ and R⁷ together are a 1,3-but-2-enedioyl group;

ii) R¹ and R² together represent =N-NR⁶ or R¹ is H and R² is -NR⁶R⁷ in which R⁶ is an organic group comprising the said functional group or is H, and R⁷ is H or R⁶ and R⁷ together are a 1,3-but-2-enedioyl group;

Gly-O is a glycosyl (saccharide) group;

n is 0 or more; and

Ac is acetyl.

8. A compound according to claim 7 in which each Gly is a sialic acid unit.

9. A compound comprising a protein with at least one free cysteine unit and, linked through a thioester bond to the side chain of the cysteine unit, with a polysialic acid, through a moiety joined at one or each terminal units of the polysialic acid.

10. A compound according to any preceding claim in which the polysaccharide has at least 2, preferably at least 10, more preferably at least 50 saccharide units, preferably sialic acid units 2,8 and/or 2,9 linked to one another.

11. A process in which a polysaccharide comprising at least one terminal unit which is derived from a sialic acid unit is reacted with a heterobifunctional reagent having a first functional group selected from N-maleimido groups, vinylsulphone groups, N-iodoacetamide groups orthopyridyl disulphide groups and a second functional group different from the first group whereby the said second functional group reacts with a terminal sialic acid derivative unit to form a covalent bond therewith and form a functional polysaccharide suitable for selective conjugation to a thiol group.

12. A process according to claim 11 in which the said second functional group is a nucleophilic group, preferably hydrazine.

13. A process according to claim 11 in which the terminal unit of the polysaccharide has a carbonyl group which reacts with said nucleophilic group.

14. A process according to claim 11 in which the said second functional group is an electrophilic group, preferably an N-alkoxycarbonyl-imide or carbodiimide moiety.

15. A process according to claim 14 in which the terminal unit of the polysaccharide has an amine group which reacts with said electrophilic group, preferably to form a peptide or a urethane linkage.

16. A process according to any of claims 11 to 15 in which the reagent comprises a bifunctional organic group linking the first and second functional groups.

17. A process according to claim 16 in which the bifunctional organic group is selected from a C₂₋₁₈-alkanediyl group, an arylene group, an oligo peptide and an oligo(alkoxy)alkyl group.

18. A process according to any of claims 11 to 17 in which the first functional group is a N-maleimide group.

19. A process according to claim 11 in which the reagent has the general formula

20 X-R-Y

in which:

X is a N-maleimido, N-iodoacetamido, S-vinylsulphonyl or S-orthopyridyldisulphide group,

25 R is alkane-diyl, arylene or aralkylene alkarylene, alkylene-oxaalkylene, or alkylene-oligooxa-alkylene or alkyl-oligopeptidyl-alkyl group; and

Y is a hydrazide, amine or N-hydroxysuccinimide group.

20. A process according to any of claims 11 to 19 in which the polysaccharide acid has at least 2, preferably at least 10, more preferably at least 50, sialic acid units, preferably 2→8 and 2→9 linked to one another.

21. A process according to any one of claims 11 to 20 in which the maleimido-functional polysialic acid is reacted with a polypeptide or a protein having at least one free unprotected Cys unit whereby the maleimide group forms a thioether linkage with the thiol group of a Cys unit to form a
5 polysialylated polypeptide or protein.

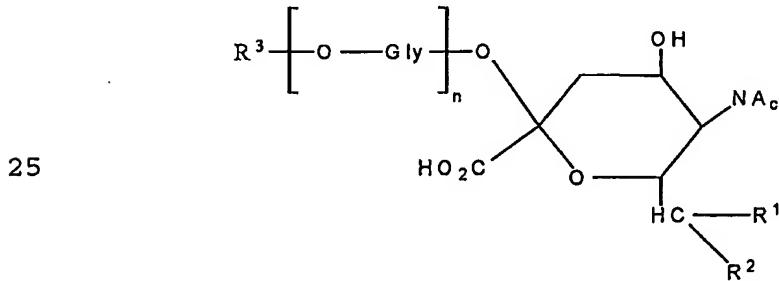
22. A process in which a compound according to any of claims 1 to 6 is reacted with a polypeptide or a protein having at least one free and unprotected Cys unit whereby the said functional group forms a thioether linkage with the thiol group of a Cys unit to form a conjugate of the
10 polysaccharide with the polypeptide or protein.

AMENDED CLAIMS

[Received by the International Bureau on 13 December 2004 (13.12.2004):
original claims 1, 4, 6, 7, 11 and 22 amended; remaining claims unchanged; (4 pages)]

CLAIMS

1. A compound comprising a polysaccharide having a pendant moiety linked to at least one terminal unit derived from a sialic acid unit which includes a functional group selected from N-maleimide groups, vinylsulphone groups, N-iodoacetamide groups and orthopyridyl disulphide groups.
- 5 2. A compound according to claim 1 in which the pendant moiety is linked at the reducing terminal unit of the polysaccharide.
3. A compound according to claim 1 or claim 2 in which the 10 moiety is linked at the non-reducing terminal unit of the polysaccharide.
4. A compound according to any preceding claim in which the moiety comprises an alkanediyl group and/or an arylene group and a linkage optionally in combination with a oxalkylene or oligooxa-alkylene group which is a secondary amine linkage, a hydrazone, an alkyl hydrazide linkage or a 15 peptide linkage.
5. A compound according to any preceding claim in which the functional group is N-maleimido.
6. A compound according to any preceding claim in which the polysaccharide is a polysialic acid, preferably consisting substantially only of 20 sialic acid units.
7. A compound according to claim 1 which has the formula



in which one of the following groups of definitions apply:

- i) R¹ is H or -CHOHCH₂OH, R² is OH and R³ is either -CH₂CHR⁴R⁵ or -CH(CH₂OH)CHR⁴R⁵ in which R⁴ and R⁵ together represent =N-NR⁶ or R⁴ is H and R⁵ is -NR⁶R⁷ in which R⁶ is an organic group

comprising the said functional group or is H, and R⁷ is H or R⁶ and R⁷ together are a 1,3-but-2-enedioyl group;

ii) R¹ and R² together represent =N-NR⁶ or R¹ is H and R² is -NR⁶R⁷ in which R⁶ is an organic group comprising the said functional group
5 or is H, and R⁷ is H or R⁶ and R⁷ together are a 1,3-but-2-enedioyl group;

Gly-O is a glycosyl (saccharide) group;

n is 0 or more; and

Ac is acetyl.

8. A compound according to claim 7 in which each Gly is a sialic
10 acid unit.

9. A compound comprising a protein with at least one free cysteine unit and, linked through a thioester bond to the side chain of the cysteine unit, with a polysialic acid, through a moiety joined at one or each terminal units of the polysialic acid.

15 10. A compound according to any preceding claim in which the polysaccharide has at least 2, preferably at least 10, more preferably at least 50 saccharide units, preferably sialic acid units 2,8 and/or 2,9 linked to one another.

11. A process in which a polysaccharide comprising at least one
20 terminal unit which is derived from a sialic acid unit is reacted with a heterobifunctional reagent having a first functional group selected from N-maleimido groups, vinylsulphone groups, N-iodoacetamide groups and orthopyridyl disulphide groups and a second functional group different from the first group whereby the said second functional group reacts with a
25 terminal sialic acid derivative unit to form a covalent bond therewith and form a functional polysaccharide suitable for selective conjugation to a thiol group.

12. A process according to claim 11 in which the said second functional group is a nucleophilic group, preferably hydrazine.

13. A process according to claim 11 in which the terminal unit of the polysaccharide has a carbonyl group which reacts with said nucleophilic group.
14. A process according to claim 11 in which the said second functional group is an electrophilic group, preferably an N-alkoxycarbonyl-imide or carbodiimide moiety.
15. A process according to claim 14 in which the terminal unit of the polysaccharide has an amine group which reacts with said electrophilic group, preferably to form a peptide or a urethane linkage.
16. A process according to any of claims 11 to 15 in which the reagent comprises a bifunctional organic group linking the first and second functional groups.
17. A process according to claim 16 in which the bifunctional organic group is selected from a C₂₋₁₈-alkanediyl group, an arylene group, an oligo peptide and an oligo(alkoxy)alkyl group.
18. A process according to any of claims 11 to 17 in which the first functional group is a N-maleimide group.
19. A process according to claim 11 in which the reagent has the general formula
20 X-R-Y
in which:
X is a N-maleimido, N-iodoacetamido, S-vinylsulphonyl or S-orthopyridyldisulphide group,
R is alkane-diyl, arylene or aralkylene alkarylene, alkylene-
25 oxaalkylene, or alkylene-oligo-oxa-alkylene or alkyl-oligopeptidyl-alkyl group;
and
Y is a hydrazide, amine or N-hydroxysuccinimide group.
20. A process according to any of claims 11 to 19 in which the polysaccharide acid has at least 2, preferably at least 10, more preferably at
30 least 50, sialic acid units, preferably 2→8 and 2→9 linked to one another.

21. A process according to any one of claims 11 to 20 in which the maleimido-functional polysialic acid is reacted with a polypeptide or a protein having at least one free unprotected Cys unit whereby the maleimide group forms a thioether linkage with the thiol group of a Cys unit to form a
5 polysialylated polypeptide or protein.

22. A process in which a compound according to any of claims 1 to 6 is reacted with a polypeptide or a protein having at least one free and unprotected Cys unit whereby the said functional group forms a thioether linkage with the thiol group of a Cys unit to form a conjugate of the
10 polysaccharide with the polypeptide or protein.